Amplified Electrochemical Detection Based on Redox Cycle at a Liquid/Liquid Interface Formed in a Microchannel

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An electrochemical detector chip with a Y-shaped microchannel was fabricated, and an amplification of the detection current based on the redox cycle at the liquid/liquid interface formed in the microchannel was demonstrated. The dependence of the amplification factor on the flow rate of the solutions was studied.

Electrochemical (EC) detection is one of suitable analytical technique for combination with microfluidic devices because of its high sensitivity and miniaturization capability of the detector and control insturments.[1–3] Further improvements of the sensitivity of EC detectors are expected because the amount of the analyte tends to be small in general when the EC detector is integrated to microfluidic devices. Recently, to improve the sensitivity in the amperometric EC detection, the current amplification method based on the redox cycle has been proposed.[4,5] After the oxidation (or reduction) at the working electrode (WE), the analyte molecules are regenerated electrochemically at the additional electrode located closely to WE and oxidized (or reduced) again at WE in this method. High amplification factor (e.g., 60-fold)[5] can be achieved by repeating the redox cycle, however, two potensiostats is required to apply different voltages to WE and the additional electrode.

In this letter, a novel current amplification method based on the redox cycle at a liquid/liquid interface formed in a microchannel was proposed. The analyte is regenerated by the spontaneous redox reaction at the interface in this method. Therefore, the amplified detection can be made simply with one potentiostat. The flow through EC detector chip with Y-shaped microchannel was fabricated, and the amplified detection of ferrocene (Fc), in an extremely small volume of sample solution was demonstrated in the flow injection analysis (FIA).

The EC detector chip was constructed from two glass substrates separated by a perfluoralkoxy (PFA) copolymer film (Figure 1). The substrates used were mechanically polished barium borosilicate glass (#7059, Corning, NY) of 0.7-mm thick. The PFA film was cut out to form a Y-shaped channel on it. The width of the channel was 460 μm, and depth of the channel was defined by the thickness of the film (25 μm). Three through-holes (about 0.5-mm diameter) as inlet and outlet ports were drilled into the upper substrate.

A WE and a counter electrode (CE) were fabricated on the bottom piece by a vacuum evaporator VE-2030 (Shinku Device Co., Ltd., Japan) as follows. First, a thin film of chromium (about 20-nm thick) as adhesion layer was deposited onto the substrate through a metal mask. Then, a gold layer (about 200-nm thick) was deposited onto the chromium. Vapor depositions were carried out at a base pressure of 7×10^{-4} Pa and at room temperature. The substrates were positioned so that only 150-μm width of WE and CE contact with the solution in the channel as shown in Figure 1b. The assembly was held together by binder clips.

Aqueous (W) and organic (Org) phase, as mobile phases, were fed to the chip with MD-1001 Baby Bee Syringe Pumps and MD-1020 Bee Hive Syringe Pump Controller (Bioanalytical Systems, Inc., West Lafayette, IN) equipped with GASTIGHT #1001 syringes (1-mL volume, Hamilton Co., Reno, NV) via fused silica capillaries. Propylene carbonate (PC) containing 0.1 M tetrabutylammonium perchlorate (TBAP) as a supporting electrolyte was used as Org. The W/PC interface was formed in the channel by feeding W and PC solutions simultaneously at the same flow rate. To stabilize the two phase stream, perpendicular part of the surface of the PFA film and upper substrate, which contact with W, was made hydrophilic by exposing to argon plasma for 30 s using a plasma ion bombarder (PIB-10, Shinku Device Co., Ltd.) before assembling. Sample solution (200 μM Fc + 0.1 M TBAP in PC) was injected to the PC mobile phase with a nano-liter injector (Injection volume: 15.7 nL, Senshu Scientific Co., Ltd., Japan). All electrochemical measurements were carried out in a two-electrode configuration. The potential, E, of WE (vs. CE which is also located in Org phase) was controlled by Model 822A Electrochemical Detector (CH Instruments, Inc., Austin, TX). The E was set at +1.0 V for oxidation of Fc in PC on the basis of the result of the hydrodynamic voltammetry performed preliminarily.

Curve 1 in Figure 2 shows the baseline-subtracted peak current, I_p, observed under the normal (non-amplifying) condition.

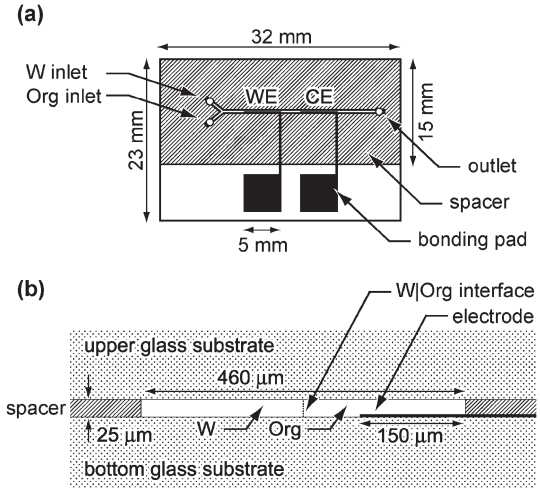

Figure 1. Schematic drawings of the micro-fabricated electrochemical detector chip: (a) the whole chip layout and (b) a cross-section view of the channel.

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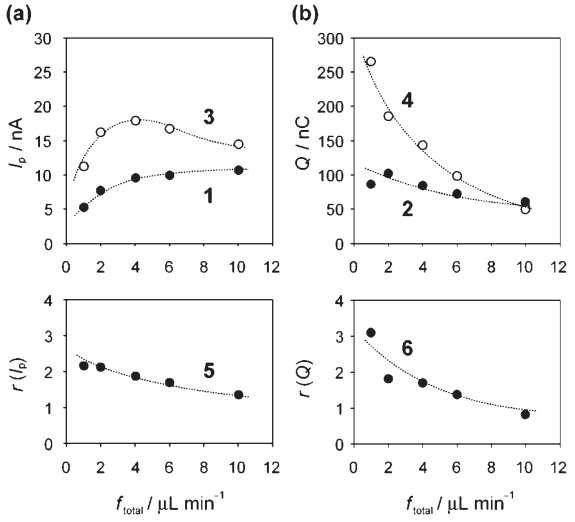

Figure 2. Dependences of the peak current, I_p, (a) and the peak area, Q, (b) observed under the normal (filled circles) and amplifying conditions (open circles) on the total flow rate, f_{total}. Curves 5 and 6 are the ratios of the value obtained under the amplifying condition to those obtained under normal condition, $r(I_p)$ and $r(Q)$, respectively. Flow rates of W and PC phases were equal, and 15.7 nL of 200 μM Fc solution was injected to PC phase in all measurements.

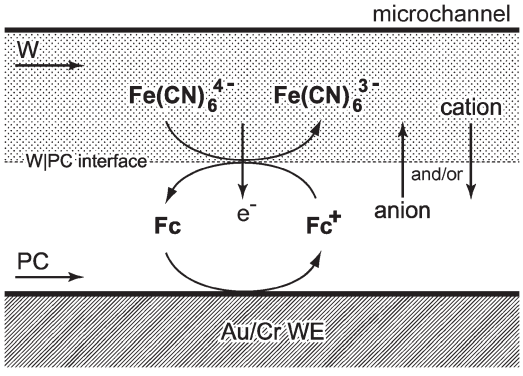

Figure 3. Schematic diagram of the mechanism of the regeneration of ferrocene by $Fe(CN)_6^{4-}$ at the W/PC interface.

In this condition, deionized water was used as W. Therefore, no redox reaction occurs at the W/PC interface. It was confirmed preliminarily that the I_p was proportional to the concentration of the Fc in the sample when up to around 200 μM. The I_p decreased with decreasing total flow rate, f_{total} (the sum of the flow rates of W and PC). The peak area (i.e., quantity of the charge electrolyzed during the measurement), Q, increased with decreasing f_{total} (curve 2) because the residence time, t, of the sample solution on WE increased with decreasing f_{total}. For example, t was about 0.35 s and about 20% of Fc in the injected sample was oxidized (i.e., the electrolysis efficiency, ε, was 20%) when f_{total} was 10 μL/min. The t and ε were about 3.5 s and 29%, respectively, when f_{total} was 1 μL/min.

Curves 3 and 4 in Figure 2 show I_p and Q observed under the same condition as that for curves 1 and 2 but in the presence of 0.1 M $K_4[Fe(CN)_6]$ in W (the amplifying condition). Both I_p and Q observed under this condition were larger than those observed under the normal condition, suggesting the regeneration of Fc by the redox reaction between oxidation product of Fc (i.e., Fc^+) and $Fe(CN)_6^{4-}$. Here, ion transfer reactions at the W/PC interface should occur simultaneously with the electron transfer (redox) reaction as shown in Figure 3 to maintain the electroneutralities in W and PC phases. The transfer of K^+ in W to PC and that of ClO_4^- in PC to W are possible in the present case.

The ratios of I_p and Q observed under the amplifying condition to those observed under the normal condition, $r(I_p)$ and $r(Q)$, were plotted as a function of f_{total}, respectively (curves 5 and 6). When f_{total} was 10 μL/min, $r(I_p)$ and $r(Q)$ were approximately one, indicating that the effect of the redox cycle was negligibly small at such a fast flow rate. Both $r(I_p)$ and $r(Q)$ increased with decreasing f_{total} and were about 2.5 at $f_{total} = 1$.

The amplification efficiency observed in this work was lower than that achieved by using the dual electrode detector.[4,5] The efficiency depends on the number of redox cycles occurring during the residence time. One approach to improve the efficiency is apparently the increment of the residence time as mentioned above. Further decrease of the flow rate and the elongation of WE in a flow direction are valid in this standpoint. The other approach is narrowing the gap between WE and the W/PC interface. The decrease of the gap reduces the time required for Fc and Fc^+ to diffuse across the PC layer (Figure 3), and accordingly the efficiency increases. In addition, WE was located to be perpendicular to the W/PC interface in the chip used. Therefore, Fc^+ produced at the region far from the W/PC interface could not take part in the redox cycle. The cycle efficiency will be improved if WE is fabricated to be parallel to the W/PC interface and close to the interface. For more quantitative discussion about the amplification efficiency, improvement of the electrode configuration from current two (WE and CE) system to three will be necessary.

In conclusion, we have demonstrated a novel technique for a sensitive electrochemical detection utilizing the two phase stream formed in a microchannel. The technique can be easily incorporated into a minituarized flow system such as micro total analysis systems (μ-TAS) without additional external devices or modification of the electrode.[6] Especially when the system is designed for multilayer flow experiment such as a solvent extraction,[7,8] the technique also can be used to monitor the extraction process. Although the case of oxidation in Org was studied in this letter, this method is also applicable to the case of reduction of an analyte in Org and oxidation/reduction of an analyte in W.

References

1 H. Suzuki, *Electroanalysis*, **12**, 703 (2000).
2 M. A. Schwarz and P. C. Hauser, *Lab on a Chip*, **1**, 1 (2001).
3 J. Wang, *Talanta*, **56**, 223 (2002).
4 S. G. Weber and W. C. Purdy, *Anal. Chem.*, **54**, 1757 (1982).
5 S. A. Brooks and R. T. Kennedy, *J. Electroanal. Chem.*, **436**, 27 (1997).
6 P. T. Radford and S. E. Creager, *Anal. Chim. Acta*, **449**, 199 (2001).
7 K. Sato, A. Hibara, M. Tokeshi, H. Hisamoto, and T. Kitamori, *Adv. Drug Delivery Rev.*, **55**, 379 (2003).
8 P.-A. Auroux, D. Iossifidis, D. R. Reyes, and A. Manz, *Anal. Chem.*, **74**, 2637 (2002).

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